Glucose and insulin dynamics associated with continuous infusion of dextrose or dextrose and insulin in healthy and endotoxin-exposed horses

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ABSTRACT

The objective of the study was to investigate and characterize the effects of a continuous rate infusion of dextrose or dextrose and insulin on glucose and insulin dynamics in both healthy and endotoxin-exposed horses. Administration of a low dose of endotoxin has been used in horses to mimic the clinicopathologic changes seen in endotoxemia, including the development of an inflammatory response. Our hypothesis was that a continuous rate infusion of insulin at a rate of 0.07 IU/kg/hr would prevent the development of hyperglycemia induced by administration of dextrose in both healthy and endotoxin-exposed horses. Nine healthy adult horses were used in the study.

In Phase 1 of the experiment, horses received a saline infusion or a dextrose infusion in a balanced crossover design. In Phase 2 of the experiment, horses received a dextrose and insulin infusion, both prior to and after receiving a low dose of endotoxin (no LPS group and LPS group respectively) in a balanced crossover design. Blood samples were collected at regular intervals throughout both phases for measurement of plasma glucose and insulin concentrations.

Infusion of dextrose alone resulted in hyperglycemia for nearly the entire study period. Insulin concentration was also increased in comparison to the saline infusion. When comparing the dextrose treatment group to the combined dextrose and insulin treatment group (no LPS group), the insulin levels were significantly greater over time in the latter group and resulted in maintenance of euglycemia. When comparing the no LPS group to the LPS group, both the glucose and insulin concentrations were higher in the LPS group but euglycemia was still achieved. These results serve to validate the dose of insulin used in this study (0.07 IU/kg/hr) in regards to effective prevention of hyperglycemia when administered concurrently with a dextrose infusion. Hyperglycemia was prevented in both healthy and endotoxin-exposed horses. In addition, the dose of insulin used was demonstrated to be safe, as hypoglycemia did not occur in any of the horses.

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Figures and Tables Appearing in the Text

List of Abbreviations

α	alpha
AIR_g	acute insulin response to glucose
ANOVA	analysis of variance
AP-1	activator protein 1
ATP-$MgCl_2$	adenosine triphosphate-magnesium chloride
β	beta
CBC	complete blood count
CD14	cellular receptor cluster of differentiation antigen 14
CGIT	combined glucose insulin test
CO_2	carbon dioxide
DI	disposition index
dL	deciliter
EDTA	ethylenediaminetetraacetic acid
EGR-1	early growth response 1
EHC	euglycemia hyperinsulinemic clamp
ELISA	enzyme linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
EPET	early phase endotoxin tolerance
FSIGT	frequently sampled intravenous glucose tolerance test
g	gram
GLUT 1	glucose transporter 1
GLUT 2	glucose transporter 2
GLUT 3	glucose transporter 3
GLUT 4	glucose transporter 4
GSK-3	glycogen synthase kinase 3
HPA	hypothalamic-pituitary-adrenal
hr	hour
ICAM-1	intercellular adhesion molecule 1
ICU	intensive care unit
I κB	inhibitor kappa B
IKK	inhibitor kappa B kinase
IL-1	interleukin 1
IL-6	interleukin 6
IL-8	interleukin 8
iNOS	induced nitric oxide synthase
IRS-1	insulin receptor substrate 1
IRS-2	insulin receptor substrate 2
IU	international units
IVGTT	intravenous glucose tolerance test
κ	kappa
kcal	kilocalorie
kg	kilogram
L	liter
LBP	lipopolysaccharide binding protein
LPET	late phase endotoxin tolerance

LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
mCD14	membrane cellular receptor cluster of differentiation antigen 14
MD2	myeloid differentiation factor 2
mg	milligram
MIRG	modified glucose to insulin ratio
mL	milliliter
MMP-2	matrix metalloproteinase 2
MMP-9	matrix metalloproteinase 9
mRNA	messenger ribonucleic acid
mU	milliunit
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor kappa B
ng	nanogram
NO	nitric oxide
O_2	oxygen
PAI-1	plasminogen activator inhibitor 1
PI 3-kinase	phosphatidylinositol 3 kinase
RISQUI	reciprocal of the square root of insulin
ROS	reactive oxygen species
sCD14	soluble cellular receptor cluster of differentiation antigen 14
SD	standard deviation
S_G	glucose effectiveness
S_I	insulin sensitivity index
TLR-4	toll like receptor 4
TNF-α	tumor necrosis factor alpha
µ	micro
µg	microgram

Chapter 1. Hyperglycemia in equine critical illness

Stress hyperglycemia is a frequent occurrence in critically ill patients in human medicine. The development of hyperglycemia is due to a state of insulin resistance and impaired glucose metabolism, a development which is mediated by increased concentrations of catecholamines, cortisol, growth hormone, glucagon, and inflammatory cytokines (Langouche and Van den Berghe 2006). Glucose production exceeds glucose clearance, with the end result being hyperglycemia. The presence of hyperglycemia has been found in critically ill human patients to be associated with significantly increased morbidity and mortality (Krinsley 2003)

Insulin resistance and systemic inflammation

During critical illness or injury, the hypothalamic-pituitary-adrenal (HPA) axis is activated and cortisol is secreted from the adrenal glands. The stress response also includes release of epinephrine, norepinephrine, growth hormone, and glucagon, as well as inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) (Marik and Raghavan 2004). One of the primary actions of these counterregulatory hormones and cytokines is the induction of hepatic insulin resistance and subsequent increased hepatic gluconeogenesis. These counterregulatory hormones act as insulin antagonists and oppose the normal inhibitory effects of insulin on lipolysis and proteolysis (Langouche and Van den Berghe 2006). The increase in lipolysis and proteolysis leads to generation of substrates for gluconeogenesis. These gluconeogenic substrates include alanine, lactate, and glycerol. Alanine is one of the main substrates, and is derived from proteolysis of skeletal muscle. Lactate is another major substrate, and hyperlactatemia is a frequent finding in critical illness. The increase in lipolysis also results

in increased availability of glycerol to be utilized by the liver in gluconeogenesis (McCowen *et al.* 2001b). The concentration of free fatty acids, which can directly inhibit the insulin signaling pathway possibly via decreased IRS-1-associated PI 3-kinase activity, also increases during lipolysis (Dresner *et al.* 1999). Hepatic gluconeogenesis is further promoted by the direct actions of glucagon. Septic patients have been found to have a marked increase in glucagon concentrations (Marik and Raghavan 2004), and in burn patients glucagon concentrations remain increased for weeks (McCowen *et al.* 2001b). In addition to increased hepatic gluconeogenesis during critical illness, hepatic glycogenolysis is upregulated with concurrent inhibition of hepatic glycogenesis. This is mediated primarily by the action of catecholamines (Vanhorebeek *et al.* 2006a).

The actions of these counterregulatory hormones and cytokines also result in development of peripheral insulin resistance. Glucose clearance can still occur in tissues that are not dependent on insulin for glucose uptake, such as the brain and red blood cells (Langouche and Van den Berghe 2006). However, in tissues such as skeletal muscle, adipose tissue, and the heart that are dependent on insulin for glucose uptake, glucose clearance is significantly decreased (Marik and Raghavan 2004). There are five known glucose transporter isoforms, three of which play a significant role in glucose uptake: GLUT 1, GLUT 2, and GLUT 4. GLUT 1 regulates basal uptake of glucose, while GLUT 2 is responsible for hepatocyte glucose uptake and release and GLUT 4 is responsible for insulin-mediated glucose uptake, which occurs in skeletal muscle, adipose tissue, and the heart (Marik and Raghavan 2004). GLUT-3 is another isoform which plays a role in insulin-independent glucose uptake, and in critical illness, along with GLUT-2, allows glucose to enter cells at a rate that is directly dependent on elevated extracellular glucose concentrations (Langouche and Van den Berghe 2006).

In tissues where GLUT 4 is active, the binding of insulin to its receptor, which is a transmembrane heterodimeric protein, results in autophosphorylation. This activates the intrinsic tyrosine kinase, which in turn leads to phosphorylation of insulin receptor substrate molecules (IRS-1 and IRS-2) and activation of phosphatidylinositol (PI) 3-kinase. The end result is translocation of GLUT 4 in vesicles from intracellular stores to the plasma membrane (McCowen *et al.* 2001b). Downstream of PI 3-kinase is a molecule known as PKB or Akt, which is activated via phosphorylation and also plays an important role in translocation of GLUT 4 transporters (Tiley *et al.* 2008). In addition, PKB also phosphorylates and inactivates a molecule known as glycogen synthase kinase-3 (GSK-3), which in turn leads to activation of glycogen synthase and promotion of glycogen synthesis.

The counterregulatory hormones associated with stress hyperglycemia inhibit these insulin signaling pathways. For example, cortisol inhibits translocation of GLUT 4 to the plasma membrane, primarily by downregulation of multiple signaling molecules (McCowen *et al.* 2001b). Growth hormone decreases the number of insulin receptors that are present and also reduces the activation of these receptors via phosphorylation of tyrosine residues. Catecholamines inhibit binding of insulin to its receptor, activation of insulin receptors, and translocation of GLUT 4 to the plasma membrane, either through a receptor or post-receptor mechanism. The end result is impairment of insulin-stimulated glucose uptake, along with impairment of glycogen synthase activity and subsequent impairment of cellular glycogen synthesis (Marik and Raghavan 2004). Consequently, insulin-stimulated glucose uptake can become severely compromised in critically ill patients. Total glucose uptake will initially be increased due to glucose uptake by tissues that are not dependent on insulin-stimulated glucose uptake (Langouche and Van den Berghe 2006). Various molecules which are produced during

critical illness, including cytokines, endothelin-1, and angiotensin II, have been demonstrated to stimulate an increase in expression and membrane localization of GLUT-1 and GLUT-3 in multiple cell types. The resulting increase in non-insulin dependent glucose uptake may overrule the normal protective downregulatory response to hyperglycemia, and result in glucose toxicity in tissues in which glucose uptake is not mediated by insulin (Langouche and Van den Berghe 2006).

Stress hyperglycemia results from a combination of factors, including increased peripheral insulin resistance and increased hepatic output of glucose (hepatic insulin resistance). There are also multiple other factors in these patients that can worsen the stress hyperglycemia. Many of these patients are on bed rest; therefore, any exercise-stimulated glucose uptake that normally would occur in skeletal muscle is absent. Even bed rest alone in healthy patients has been shown to decrease skeletal muscle sensitivity to insulin (Stuart *et al.* 1988). These patients are also frequently receiving some form of parenteral or enteral nutrition. Administration of densely caloric parenteral or enteral nutrition can contribute to the development of hyperglycemia (Krinsley 2003). For example, a study conducted in nondiabetic patients who were on total parenteral nutrition found that there was a 50% likelihood that those who received dextrose at rates greater than 4 mg/kg/min would develop hyperglycemia. At rates below this, the likelihood of hyperglycemia was significantly lessened (Rosmarin *et al.* 1996). Dialysis solutions can also be a source of dextrose in intensive care unit patients. Intensive care unit patients may also be receiving corticosteroids, which will further promote development of an insulin resistant state.

Sepsis is also a common finding in critically ill patients, and is frequently associated with development of insulin resistance. During sepsis, tyrosine phosphorylation of IRS-1 is inhibited,

resulting in decreased translocation of GLUT 4 to the plasma membrane. The inflammatory cytokine TNF-α induces serine phosphorylation of IRS-1, which causes IRS-1 to have a reduced affinity for the insulin receptor. Thus, IRS-1 is unable to be tyrosine phosphorylated by the insulin receptor and so subsequently cannot bind PI-3-kinase. This effect of TNF-α has been demonstrated in endothelial cells, hepatocytes, and adipocytes (McCowen *et al.* 2001b).

Nuclear factor-kappa B (NF-κB) is a nuclear transcription factor that plays a significant role in the induction of pro-inflammatory mediators. In its inactivated state, it is normally bound to inhibitor κB (I κB). As a result of this binding, it remains localized to the cytosolic compartment. Activation of NF- κB is mediated by a serine kinase known as inhibitor κB kinase (IKK). IKK itself is activated by the binding of lipopolysaccharide (LPS) to the Toll-like receptor 4 (TLR4) or via the effects of TNF- α or IL-1. Once activated, serine phosphorylation of I κB results in its destruction and the subsequent nuclear translocation of NF- κB. IKK also serine phosphorylates IRS-1, which is another possible mechanism for the insulin resistance seen in pro-inflammatory states (Marik and Raghavan 2004).

Glucose toxicity

Glucose itself has been shown to have strong pro-inflammatory effects. Glucose has been found to be associated with increased concentrations of intranuclear NF-κB and TNF-α, as well as a decrease in the amount of cytosolic I κB (Guha *et al.* 2000). The presence of glucose also leads to increased activation of other pro-inflammatory transcription factors, including early growth response-1 (EGR-1) and activator protein-1 (AP-1) (Dandona *et al.* 2005). These, along with NF-κB, regulate the expression of inflammatory mediators such as TNF-α, IL-6, matrix metalloproteinases (MMP's), IL-8, tissue factor, and tissue plasminogen activator inhibitor-1.

In diabetic patients, hyperglycemia has been shown to enhance oxidative stress, with mechanisms including increased mitochondrial superoxide production and lipid peroxidation (Langouche and Van den Berghe 2006; Marik and Raghavan 2004). Administering oral glucose to healthy individuals has been shown to promote generation of reactive oxygen species (ROS) by leukocytes (Marik and Raghavan 2004; Mohanty *et al.* 2000). Generation of this increased oxidative stress can occur in normal individuals even without glucose levels reaching a pathological range and with normal endogenous insulin secretion being present (Dandona *et al.* 2005; Dhindsa *et al.* 2004). Administering oral glucose has furthermore been shown to increase plasma IL-8 levels; the state of hyperglycemia itself leads to an increase in expression of the IL-8 gene (Chettab *et al.* 2002; Marik and Raghavan 2004; Straczkowski *et al.* 2002). IL-8 is generally considered to be a pro-inflammatory cytokine and is chemotactic for neutrophils. Other pro-inflammatory effects of glucose include generation of a pro-thrombotic state, increased expression of matrix metalloproteinases, and reduction of endothelial nitric oxide levels (Marik and Raghavan 2004). Conversely, the prevention of hyperglycemia prevents and/or reverses mitochondrial damage in hepatocytes in critically ill patients (Vanhorebeek *et al.* 2005a).

Pro-inflammatory mediators produced in critical illness, such as various cytokines, endothelin-1, and vascular endothelial growth factor, all increase expression and membrane localization of the glucose transporters GLUT-1 and GLUT-3, which regulate non-insulin dependent glucose transport. This upregulation during inflammation supersedes the normal physiological downregulation of these receptors that should occur in order to protect against hyperglycemia. Therefore, it is likely that tissues, such as the liver and brain, which do not depend on insulin for glucose uptake, are particularly susceptible to glucose toxicity due to cellular glucose overload. In fact, this toxicity may develop in any cell type that relies on non-

insulin dependent glucose transporters, such as hepatocytes, renal tubular cells, endothelial, epithelial, and immune cells, pancreatic β cells, and gastrointestinal mucosa (Vanhorebeek *et al.* 2005b).

Effects of hyperglycemia in the critically ill human patient

Hyperglycemia at admission and throughout the hospital stay has been associated with increased mortality and morbidity in hospitalized human patients. For example, a prospective study looking at non-diabetic trauma patients admitted to the intensive care unit over a 2 year period found that admission hyperglycemia was associated with an overall greater infection rate and length of hospital stay, and that the patients with hyperglycemia at admission had a 2.2-times greater risk of mortality when adjusted for age and Injury Severity Score (Sung *et al.* 2005). A retrospective study examining 1826 intensive care unit patients with a wide range of medical and surgical diagnoses found that even a modest degree of hyperglycemia resulted in a significantly increased mortality rate (Krinsley 2003). This effect has been demonstrated in various sub-populations of patients, including those with acute myocardial infarction, severe brain injury, trauma patients, and pediatric critically ill patients, as well as for hyperglycemia present at admission and persistent hyperglycemia throughout hospitalization (Bochicchio *et al.* 2005a; Bochicchio *et al.* 2005b; Jeremitsky *et al.* 2005; Laird *et al.* 2004; Rovlias and Kotsou 2000; Sala *et al.* 2002; Yendamuri *et al.* 2003). Furthermore, patients with hyperglycemia are more prone to developing infections, as hyperglycemia can lead to a decrease in immune function (Marik and Raghavan 2004). For example, hyperglycemia has been shown to decrease polymorphonuclear neutrophil function and inhibit intracellular bactericidal and opsonic activity (Langouche and Van den Berghe 2006; Vanhorebeek *et al.* 2005b, 2006a). In addition, polymorphonuclear leukocyte and alveolar macrophage respiratory burst is inhibited by

hyperglycemia (Marik and Raghavan 2004; McCowen *et al.* 2001b). Hyperglycemia also suppresses the immune system by glycosylation of immunoglobulins, which results in their inactivation (Langouche and Van den Berghe 2006). Another detrimental effect of hyperglycemia is increased protein catabolism, although the exact mechanisms for this are unknown (McCowen *et al.* 2001b).

The detrimental effects of hyperglycemia are not as well described in the horse. One retrospective study examining prognostic variables in horses with colic found that higher blood glucose levels were associated with a decreased probability of survival (Parry *et al.* 1983). In another study assessing horses with acute abdominal disease, it was found that horses that did not survive to hospital discharge had higher mean blood glucose concentrations at admission as well as 24, 36, and 48 hours after admission (Hollis *et al.* 2007). These horses also had higher maximum and minimum blood glucose concentrations during the first 24 hours of admission.

Insulin therapy

Insulin, in contrast to glucose, has considerable anti-inflammatory properties. One of the most prominent examples of this is the fact that insulin suppresses the pro-inflammatory transcription factors NF-κB, EGR-1, and AP-1. These transcription factors regulate the genes for expression of various substances found during inflammation, including MMP-2, MMP-9, tissue factor (TF), monocyte chemoattractant protein (MCP) -1, ICAM-1, and plasminogen activator inhibitor-1 (PAI-1) (Dandona *et al.* 2005). It has been shown that an infusion of insulin causes a decrease in intranuclear NF-κB levels and an increase in IκB levels. As NF-κB is responsible for the transcription of the genes encoding these pro-inflammatory mediators, it stands to reason that a decrease in intranuclear NF-κB concentrations would also lead to a decrease in the levels of the

pro-inflammatory mediators themselves, and this effect has been demonstrated (Dandona *et al.* 2001). Insulin also has antioxidant properties, and suppresses the generation of reactive oxygen species (Dandona *et al.* 2001; Dandona *et al.* 2005). The enzyme NADPH oxidase is responsible for the generation of the superoxide radical from molecular oxygen. Levels of $p47^{phox}$ subunit, the major protein of this oxidase complex, have been found to decrease with administration of an insulin infusion (Dandona *et al.* 2001).

Insulin also induces upregulation of nitric oxide synthase activity in the endothelium and in platelets and increases production of nitric oxide (NO), thereby promoting vasodilation and inhibiting the aggregation of platelets (Dandona *et al.* 2005). The specific isoform of nitric oxide synthase that is upregulated is known as eNOS and is constitutively expressed, primarily in the endothelium but also hepatocytes and muscle cells. In contrast, insulin has been shown to suppress the expression of another isoform of nitric oxide synthase known as iNOS in the liver and skeletal muscle (Langouche *et al.* 2005). iNOS expression is induced by a variety of stimuli, including the presence of inflammation, and leads to high concentrations of NO being generated. Low concentrations of NO, which are generated by constitutive expression of eNOS, exert anti-inflammatory and anti-adhesive effects. However, high concentrations of NO, generated by increased activity of iNOS, exert proinflammatory effects.

Another benefit of insulin is an inhibitory effect on lipolysis, thus decreasing the amount of free fatty acids that are produced. This in turn reduces the prothrombotic state induced by the presence of free fatty acids (Dandona *et al.* 2001). Administration of insulin also aids in reversal of hypertriglyceridemia and general correction of dyslipidemia (Vanhorebeek *et al.* 2005b). Insulin has also been shown to have an anti-inflammatory effect through suppression of the hepatic acute phase response, as indicated by decreased levels of C-reactive protein (Hansen *et*

al. 2003). C-reactive protein is an acute phase response protein produced by the liver that acts as a marker of the degree of inflammation that is present. C-reactive protein likely binds to ligands found on the surface of bacteria and necrotic tissue and plays a role in the activation of the complement system and leukocytes. The reduction of C-reactive protein concentrations by insulin appears to be linked to a reduction in mortality (Hansen *et al.* 2003).

Strict glycemic control

Parenteral nutrition is a supportive therapy that is commonly required in critically ill human patients. A concurrent insulin infusion is often required for the strict maintenance of euglycemia. Traditionally, effective glycemic control was regarded as maintaining the blood glucose level below 180-200 mg/dL. More recently, the increasingly widespread trend has been to achieve strict normalization of blood glucose levels, in which blood glucose level is kept under much tighter control (Langouche and Van den Berghe 2006). Many studies have demonstrated the numerous advantages gained by using intensive insulin therapy to maintain strict glycemic control. A landmark prospective study of 1,548 patients in a surgical critical care unit showed a 34% reduction in overall in-hospital mortality when maintaining the blood glucose between 80-110 mg/dL (van den Berghe *et al.* 2001). Furthermore, a significant decrease in critical care complications occurred, including a reduction in bloodstream infections, bacteremia, acute renal failure requiring dialysis or hemofiltration, critical illness polyneuropathy, dependence on mechanical ventilation, and prolonged inflammation. Similar benefits have been appreciated for glucose management protocols which require even less tight control of blood glucose. A study looking at a heterogeneous population of critically ill adult patients found a

reduction in hospital mortality of 29.3% and length of ICU stay of 10.8% when blood glucose was maintained lower than 140 mg/dL (Krinsley 2004). Other benefits included a decreased incidence of new renal insufficiency and decreased requirement for red blood cell transfusions. A smaller prospective, randomized study investigating blood glucose control between 80 to 120 mg/dL in a surgical ICU found a significant reduction in total nosocomial infections with the stricter blood glucose control regimen (Grey and Perdrizet 2004). In a study investigating the effects of stricter glycemic control in critically ill patients in a medical ICU, in which sepsis and/or septic shock were common findings, the use of intensive insulin therapy was significantly associated with reduced morbidity in all the ICU patients and reduced morbidity and mortality among patients who were in the ICU for three or more days (Van den Berghe *et al.* 2006).

Insulin therapy and glycemic control have also been found to have specific benefits in various subpopulations of critically ill patients. For example, a study looking at patients with isolated brain injury found that intensive insulin therapy reduced the risk of critical illness polyneuropathy and ventilation dependency, as well as reducing the incidence of seizures and diabetes insipidus (Van den Berghe *et al.* 2005). In addition, mean and maximal intracranial pressures were reduced, with concurrent maintenance of adequate cerebral perfusion pressures with eightfold less vasopressors. Furthermore, long term rehabilitation of these patients was achieved, with more patients in the intensive insulin control group being able to care for most of their own needs independently at 12 months follow up. Insulin therapy has also been shown to promote improvement of myocardial function and protection of the myocardium during acute myocardial infarction and open heart surgery (Das 2003). Mechanisms for this include normalization of blood glucose concentrations, but also direct anti-apoptotic properties of insulin which are separate from glycemic control. Intensive insulin therapy also results in protection of

the hepatocellular mitochondria in critically ill patients and prevents development of severe mitochondrial ultrastructural abnormalities typically seen in patients receiving conventional insulin therapy (Vanhorebeek *et al.* 2005a).

The role of intensive insulin therapy in sepsis is not as well-defined. Current guidelines in human medicine for management of severe sepsis and septic shock recommend the use of a validated protocol for insulin dose adjustments with the aim of keeping blood glucose concentrations in the <150 mg/dL range (Dellinger *et al.* 2008). A study examining the use of intensive insulin therapy (to maintain blood glucose between 80 to 110 mg/dL) in patients with severe sepsis found no difference between the conventional therapy group and the intensive therapy group in regards to mortality rate at 28 days or the mean score for organ failure (Brunkhorst *et al.* 2008). Furthermore, the rates of severe hypoglycemia (blood glucose ≤ 40 mg/dL) and serious adverse events associated with hypoglycemia were significantly higher in the intensive therapy group in comparison to the conventional therapy group.

It appears that intensive insulin therapy prevents development of hyperglycemia primarily through increased glucose uptake by skeletal muscle rather than affecting hepatic management of glucose (Vanhorebeek *et al.* 2005b). It has been found that mRNA levels of GLUT 4 and hexokinase-II, the rate-limiting enzyme in intracellular insulin-stimulated glucose metabolism, are increased in skeletal muscle after intensive insulin therapy (Mesotten *et al.* 2004). In contrast, mRNA levels of glucokinase, the rate-limiting enzyme for insulin-stimulated glucose uptake, remained unchanged in the liver after intensive insulin therapy. Furthermore, mRNA levels of phosphoenolpyruvate carboxykinase, the rate-limiting enzyme for gluconeogenesis, were not decreased in the liver by insulin therapy. Concentrations continued to remain elevated in critically ill patients despite insulin therapy (Mesotten *et al.* 2002).

The prevention of hyperglycemia, rather than the infused insulin dose *per se,* appears to be largely responsible for many of the benefits seen with intensive insulin therapy, including reduction of mortality, critical illness polyneuropathy, inflammation, and bacteremia (Van den Berghe *et al.* 2003). Furthermore, in the same study, tight glucose control (blood glucose < 110 mg/dL) was required in order to best achieve the aforementioned reductions in complications. Even a moderate degree of hyperglycemia (110-150 mg/dL) was associated with a higher likelihood of these complications occurring.

The use of intensive insulin therapy to achieve strict glycemic control is becoming increasingly well-established as a requirement in critically ill patients. Many hospitals have begun utilizing nomograms in order to standardize the administration of insulin therapy. A nomogram consists of a carefully designed protocol that is to be followed by hospital staff to adjust the rate of insulin infusion, with the adjustment based both on a patient's current blood glucose level and current rate of insulin infusion. This is in contrast to the more conventional approach, in which the clinician arbitrarily decides if and to what extent the insulin infusion should be adjusted. Recent studies have shown that standardization of intensive insulin therapy through the use of nomograms leads to significantly improved glycemic control, both by decreasing the time to reach the target glucose range and by increasing the time spent within the target range. Furthermore, nomograms have been demonstrated to increase safety by reducing the incidence of severe hypoglycemia and the frequency that "rescue" dextrose must be administered or that the insulin infusion must be temporarily discontinued (Brown and Dodek 2001; Dilkhush *et al.* 2005; Goldberg *et al.* 2004; Kanji *et al.* 2004; Krinsley 2004). The majority of the described algorithms utilize continuous infusions of insulin, although subcutaneous administration has also been utilized in some protocols. A study looking at 3554 patients with

diabetes undergoing coronary artery bypass grafting found that using a continuous insulin infusion resulted in a significantly lower mortality versus using a subcutaneous insulin administration protocol and that using a continuous insulin infusion was independently protective against death (Furnary *et al.* 2003). Furthermore, significantly better glycemic control was achieved with a continuous insulin infusion in contrast to using subcutaneous insulin. A review by Meijering *et al.* assessed 24 studies examining insulin algorithms and found that using subcutaneous insulin as part of the protocol resulted in glycemic control in only two-thirds of ICU patients (Meijering *et al.* 2006). The majority of the studies that were examined utilized continuous intravenous insulin infusions along with intravenous bolus injections. They found that glycemic control in critically ill patients was best accomplished using a continuous infusion of insulin combined with frequent blood glucose measurements and the use of the last two blood glucose readings to make changes as needed in the insulin infusion rate. Furthermore, they found that using a dynamic sliding scale, in which the dosage of insulin is changed according to the blood glucose level, achieved better results than using a fixed sliding scale, in which a fixed amount of insulin is administered based on the blood glucose level.

Insulin resistance in the horse

Insulin resistance is defined as a reduced response to insulin by insulin-sensitive cells, mainly in muscle, adipose tissue, and the liver (Kronfeld *et al.* 2005b). Possible mechanisms include diminished insulin signaling at the cell surface (low insulin sensitivity), for example, due to a decrease in number of insulin receptors present or a decrease in the affinity of the insulin receptor for insulin, or alternatively, deficiencies in intracellular signaling (insulin

ineffectiveness). Insulin resistance has been reported in the horse in relation to a variety of conditions and factors, including pituitary adenoma, obesity, diet, reproductive status, and endotoxemia (Fowden *et al.* 1984; Hoffman *et al.* 2003; Kronfeld *et al.* 2005a; Kronfeld *et al.* 2005b; Treiber *et al.* 2005a).

Pars intermedia pituitary adenoma, or Equine Cushing's disease, is thought to lead to development of an insulin resistant state due to excess circulating concentrations of cortisol (McCue 2002). Cortisol antagonizes the actions of insulin, which results in decreased uptake of glucose in peripheral tissues, increased hepatic gluconeogenesis, and decreased glycogen synthesis. In rat models, corticosteroid administration has been shown to decrease insulin-stimulated activation of IRS-1 and PI 3-kinase, as well as translocation of GLUT 4 transporters to the cell membrane (Brown *et al.* 2007; Dimitriadis *et al.* 1997; Saad *et al.* 1993). Exogenous corticosteroid administration also results in development of insulin resistance. Tiley *et al.* (2007) found significant insulin resistance in a group of healthy horses after 21 days of dexamethasone administration. Associated with this development of insulin resistance was decreased GSK-3 phosphorylation in skeletal muscle, which may have further explained the concurrent absence of insulin-stimulated increase in glycogen synthase fractional activity that was also seen in these horses (Tiley *et al.* 2008). Interestingly, administration of a single dose of hydrocortisone to normal horses actually resulted in an increase in sensitivity of peripheral tissues to exogenous insulin (de Graaf-Roelfsema *et al.* 2005). In the same study, prolonged administration of equine growth hormone resulted in decreased sensitivity of peripheral tissues to exogenous insulin. Another study looking at a population of nonobese ponies predisposed to pasture-associated laminitis found that administration of dexamethasone (via an overnight dexamethasone suppression test) resulted in a significantly exaggerated increase in serum insulin concentrations

in comparison to the control group of ponies (Bailey *et al.* 2007). These ponies were also documented to have compensated insulin resistance based on results of a frequently sampled intravenous glucose tolerance test and minimal modeling.

A newer syndrome, known as the equine metabolic syndrome, or peripheral Cushing's syndrome (as opposed to conventional Cushing's syndrome, which is due to a pituitary adenoma), also involves the development of insulin resistance and is at least to some extent comparable to human metabolic syndrome, which is defined by insulin resistance, hypertension, and dyslipidemia (Johnson 2002). These horses are typically obese, prone to episodes of laminitis, and generally regarded as "easy keepers." In humans, it has been shown that the presence of omental adiposity contributes to metabolic syndrome, as omental adipocytes are metabolically active and lead to generation of mediators such as free fatty acids, cortisol, leptin, and resistin, which in turn may promote development of insulin resistance and an obese phenotype. Omental adipocytes also have an enzyme known as 11-β hydroxysteroid dehydrogenase, which promotes conversion of cortisone to cortisol, thus leading to further development of insulin resistance. It is hypothesized that similar processes may be occurring in horses with equine metabolic syndrome (Johnson 2002). Obesity in general in horses has been shown to be associated with reduced insulin sensitivity. One study found that insulin sensitivity was approximately 80% lower in obese horses versus nonobese horses (Hoffman *et al.* 2003). It also appeared that obese horses were primarily dependent on glucose-mediated glucose disposal based on a higher glucose effectiveness value, which is an assessment of the ability of glucose to regulate its own disposal rather than relying on insulin. In addition, obese geldings had increased secretion of insulin, which was likely in response to decreased insulin sensitivity. A significant

association between obesity, insulin resistance, and increased concentrations of inflammatory cytokines has been demonstrated in the horse (Vick *et al.* 2007).

A prelaminitic metabolic phenotype has also been identified in laminitis-prone ponies (Bailey *et al.* 2008). This phenotype was characterized by insulin resistance, elevated plasma triglyceride levels, and hypertension, and was apparent when the ponies were on summer pasture but not during the winter. Insulin resistance in general is thought to contribute to development of laminitis via multiple mechanisms, including impairing glucose delivery to hoof keratinocytes and/or changing vascular dynamics and blood flow to the hoof (Frank 2006). It has been shown in hoof explants that glucose deprivation leads to separation at the epidermal-dermal interface (Pass *et al.* 1998). In addition, the presence of GLUT 4 transporters has been demonstrated in equine keratinocytes (Mobasheri *et al.* 2004). This supports the theory that glucose uptake in the equine hoof is mediated at least to some extent by insulin. Insulin, independent of changes in blood glucose concentration, may also play a role in the induction of laminitis. A study in ponies in which prolonged hyperinsulinemia was maintained with concurrent euglycemia resulted in the development of laminitis (Asplin *et al.* 2007).

Diet also plays an important role in insulin resistance. In the same study by Hoffman *et al.*, it was found that horses fed a diet rich in starch and sugar had decreased insulin sensitivity in comparison to horses fed a diet rich in fiber and fat. Another study similarly found a decrease in insulin sensitivity of 37% in Thoroughbred weanlings fed a diet rich in starch and sugar in comparison to those fed a diet rich in fat and fiber (Treiber *et al.* 2005a). The decrease in insulin sensitivity was compensated for by increased insulin secretion, reflecting an adaptation of these horses to a high-glycemic index feed.

Pregnancy in the mare, as in other species, results in significant changes in carbohydrate metabolism, including some degree of insulin resistance, increased insulin degradation, and increased pancreatic β cell sensitivity to both endogenous and exogenous glucose (Fowden *et al.* 1984). It is suggested that the reduced effectiveness of insulin in late pregnancy serves to shunt more glucose towards the fetus and decreases maternal glucose uptake and utilization. Placental uptake of glucose is dependent primarily on the glucose concentration gradient rather than on insulin. Peripheral antagonism of insulin may be mediated by increased levels of free fatty acids as well as various hormones associated with pregnancy such as progesterone.

Maternal diet during gestation can also influence the insulin sensitivity of the foal. One study demonstrated reduced insulin sensitivity in foals at 160 days of age when their dams had been fed a diet high in soluble carbohydrates during late gestation (George *et al.* 2007). Newborn foals in this study were demonstrated to be very insulin sensitive, but in another study (Holdstock *et al.* 2004) were thought to be somewhat insulin resistant, as demonstrated by a deficient clearance of glucose in response to exogenous glucose administration

Endotoxemia and insulin resistance in the horse

Endotoxemia in the horse has been demonstrated to induce a transient insulin resistance. Endotoxin is a heat-stable lipopolysaccharide freed from the outer membrane of the cell wall of gram-negative bacteria due to lysis of the bacteria or rapid bacterial growth. In the horse, the most common causes of endotoxemia are gastrointestinal abnormalities such as colic or colitis which result in increased intestinal wall permeability and subsequent leakage of endotoxin (Toth *et al.* 2008; Werners *et al.* 2005). Exogenous sources of LPS, such as from gram-negative

bacteremia (i.e. pleuropneumonia, retained fetal membranes, metritis, infected wounds, neonatal septicemia, peritonitis) can also result in development of endotoxemia.

Endotoxin (lipopolysaccharide, LPS) is made up of an inner hydrophobic lipid A moiety, a core oligosaccharide, and an outer O-specific polysaccharide (Werners *et al.* 2005). The lipid A moiety is highly conserved among gram-negative bacteria, and is the component of lipopolysaccharide which binds to LPS-binding protein (LBP) in the plasma, initiating a cascade of events which results in endotoxic shock (see figure 1). After binding to LBP, the LPS-LBP complex binds to a glycoprotein known as CD14 (cellular receptor cluster of differentiation antigen 14) on the cell surface of monocytes and macrophages. CD14 can be present in both a membrane bound form (mCD14) as well as a soluble form (sCD14). The LPS-CD14 complex then interacts with a transmembrane receptor known as the Toll-like receptor 4 (TLR4). TLR4, along with another protein complex located on the cell surface known as myeloid differentiation factor 2 (MD2), then interacts with various cytosolic adaptor proteins to result in activation of two major pathways: the NFκB pathway and the mitogen-activated protein kinase (MAPK) pathway. Both pathways result in increased DNA transcription and production of various inflammatory mediators, including cytokines such as TNF-α, IL-6, and IL-1 (Werners *et al.* 2005). These inflammatory mediators are believed to contribute to insulin resistance, and have been linked to development of an insulin resistant state in human patients in cases of burns, trauma, and infections (McCowen *et al.* 2001b).

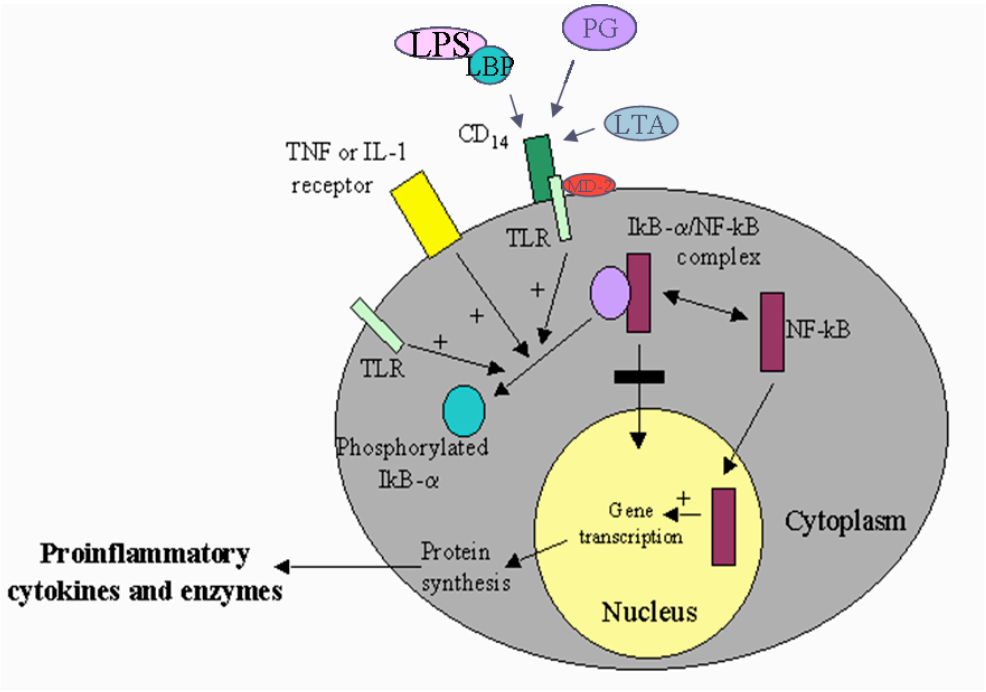

Figure 1. Cellular receptors responsible for the initiation of a pro-inflammatory response and the NF-kB pathway leading to production of pro-inflammatory mediators. (Courtesy of H. C. McKenzie III)

Prolonged endotoxemia has been found to cause changes in the early steps of the insulin-signaling cascade in liver and skeletal muscle in a rat model involving infusion of exogenous endotoxin to create clinical low grade sepsis (McCowen *et al.* 2001a). Changes seen included decreased tyrosine phosphorylation of insulin receptors and IRS-1 and IRS-2, decreased IRS-1 associated PI 3-kinase activity, and decreased concentrations of insulin receptor and IRS proteins. Administration of LPS has also been used in humans to create a human model replicating the metabolic changes that are frequently seen in sepsis and critical illness, including development of acute insulin resistance and a febrile systemic inflammatory response with concomitant hormonal and cytokine changes (Agwunobi *et al.* 2000). Administration of 20 U/kg

Escherichia coli endotoxin to healthy human subjects in one study resulted in development of fever, tachycardia, and mild arterial hypotension (Agwunobi *et al.* 2000). Insulin resistance developed, as indicated by decreased glucose utilization in a euglycemic hyperinsulinemic clamp test, and was attributed to impaired nonoxidative glucose disposal rather than abnormal glucose oxidation. In addition, there were significant increases in hormones with insulin antagonistic effects, including cortisol, growth hormone, and glucagon. Plasma TNF-α and IL-6 levels also increased after LPS infusion.

Similar models of endotoxemia-induced metabolic derangements have been developed in the horse. Intravenous administration of high dose endotoxin (50-200 μg/kg) has been demonstrated to cause collapse, severe hypotension, respiratory distress, and death in horses (Clark and Moore 1989). It has become more common to use low dose endotoxin administration to mimic the clinicopathologic changes seen in endotoxemia without inducing systemic shock and possible death. Administration of 30 ng/kg endotoxin as a slow infusion in one study resulted in significant increases in temperature, heart rate, and respiratory rate, as well as development of depression and injected mucous membranes. Signs of colic also developed in some of the horses (Clark and Moore 1989). In another study performed by the same group of researchers, endotoxin infusion at the same dose resulted in significant increases in average pulmonary arterial pressure as well as a trend toward increased diastolic and systolic pulmonary arterial pressure and pulmonary vascular resistance (Clark *et al.* 1991). Administration of 35 ng/kg of endotoxin in another study resulted in significant increases in temperature and heart rate, mild depression, and changes in mucous membrane color and capillary refill time (Bueno *et al.* 1999). In addition, there was a significant increase in mean arterial pressure, mean pulmonary arterial pressure, arterial pH, and hematocrit, as well as a significant decrease in arterial PCO_2

and PO_2. There was an initial decrease in leukocytes and segmented neutrophils below baseline from 1-2 hours after LPS administration, followed by an increase in both values above baseline values from 5-24 hours after LPS administration. There was also a significant increase in serum IL-6 and serum TNF-α concentrations from 1 to 8 hours and 1 to 2 hours after LPS administration respectively. Other changes that have been found with similar doses of endotoxin include a mild transient leukopenia without a neutropenia, a transient significant increase in total protein, albumin, globulin, calcium, and potassium concentrations, a decrease in creatinine and phosphorus concentrations, and a significant increase in chloride concentration over time (Tetens *et al.* 2004). Additionally, ionized calcium, ionized magnesium, and urinary fractional excretion of calcium, magnesium, and potassium were found to decrease, along with an increase in urinary fractional excretion of phosphorus and sodium (Toribio *et al.* 2005). In this study, serum parathyroid hormone (PTH) concentrations increased significantly, but this was not a consistent finding in all the horses. Insulin also increased significantly after endotoxin infusion; however, insulin concentrations were only measured in a subpopulation of the study group. Blood work (CBC, serum chemistry profile) and urine profiles and analyses returned to normal 48 hours after infusion of endotoxin in all horses in this study. The low-dose endotoxin model (20-35 ng/kg), although associated with some variation in clinicopathologic changes, in general produces predictable and reproducible changes in hematologic, hemodynamic, and physical variables, with data that is comparable among various studies (Winchell *et al.* 2002). This model has been successfully used in studies to test the efficacy of various pharmacologic agents, such as flunixin meglumine, ATP-$MgCl_2$, and TNF-α antibody, on ameliorating typical changes seen with endotoxemia (Barton *et al.* 1998; Danek 2006; Tetens *et al.* 2004).

A low-dose endotoxin model has also been used in the horse to investigate changes in insulin and glucose dynamics in response to endotoxin administration (Toth *et al.* 2008). It was found that insulin sensitivity was decreased at 24 hours after endotoxin challenge, and that pancreatic β cell secretion of insulin was compensatorily increased. Another study also using a low-dose endotoxin model to investigate insulin resistance induced by systemic inflammation found a transient increase in insulin sensitivity at 6 hours after LPS administration, followed by development of insulin resistance at 24 hours after LPS administration (Vick *et al.* 2008). This biphasic response is commonly seen in other species, including humans; however in humans the initial increase in insulin sensitivity is typically seen within 2 hours (Agwunobi *et al.* 2000). This initial increase may be due to cytokine-stimulated non-insulin-mediated glucose uptake (Vick *et al.* 2008). This study also demonstrated that endotoxin challenge, mimicking a systemic inflammatory response, resulted in increased mRNA expression of the inflammatory cytokines IL-1, IL-6, and TNF-α in adipose tissue.

Repeated administration of LPS can lead to a phenomenon known as endotoxin tolerance. Endotoxin tolerance occurs in two stages: early phase endotoxin tolerance (EPET) and late phase endotoxin tolerance (LPET) (Allen *et al.* 1996). EPET, which is a transient phenomenon that occurs within hours to days and does not involve production of antibodies to endotoxin, has been demonstrated in the horse using a dose of 50 ng/kg LPS administered 24 hours apart (Allen *et al.* 1996). Altered host responses after the second dose of endotoxin included a decreased duration of fever and attenuated TNF-α response.

Assessing insulin resistance in the horse

Basal glucose and insulin measurements can be assessed in the horse but are not very accurate indicators of insulin resistance, as they can vary fairly dramatically in an individual from moment to moment based on multiple factors such as stress, feeding, and normal diurnal variation (Firshman and Valberg 2007). Fasting hyperglycemia can be indicative of a decreased pancreatic β cell insulin secretion or of peripheral tissue resistance to insulin. Fasting hyperinsulinemia can suggest an increased compensatory secretion of insulin by the pancreatic β cells due to peripheral tissue resistance to insulin. Glucose–to-insulin and insulin-to-glucose ratios can also be used to assess glucose and insulin dynamics in horses. The former evaluates the effect of insulin on glucose while the latter evaluates the effect of glucose in stimulating pancreatic secretion of insulin (Firshman and Valberg 2007).

Glucose tolerance tests can be either intravenous or oral. In an intravenous glucose tolerance test (IVGTT), a high dose of glucose in the form of dextrose (0.5 g/kg as a 50% solution) is administered intravenously after a 10 to 12 hour fast. Blood samples are collected regularly until 5 to 6 hours after injection for glucose and insulin measurements. A normal response is for the glucose concentration to peak in 15 minutes and for insulin to peak at 30 minutes. Blood glucose should return to baseline level in 1 hour (Ralston 2002). An increased glucose peak and/or delayed glucose curve can indicate impaired pancreatic β cell secretion of insulin, decreased glucose- or insulin-stimulated glucose uptake, or fat adaptation which has a glucose sparing effect (Kronfeld *et al.* 2005a). An impairment of insulin secretion by the pancreatic β cells would be suggested by a blunted and/or delayed insulin response (Firshman and Valberg 2007). Increased glucose clearance, such as occurs in cases of polysaccharide storage myopathy, would suggest increased insulin sensitivity.

An oral glucose tolerance test, in addition to the factors assessed by the IVGTT, also evaluates gastric emptying and intestinal absorption of glucose (Ralston 2002). The test involves administration via nasogastric tube of 1 g/kg dextrose as a 20% solution after a 12 to 16 hour fast. Blood samples are drawn for glucose and insulin measurements for 6 hours after dextrose administration. Glucose levels typically peak at 90 to 120 minutes and should return to baseline within 4 to 6 hours. Insulin peaks at 1.5 to 2.5 hours. A decreased glucose response can indicate increased insulin sensitivity, delayed gastric emptying, or reduced intestinal absorption, while an increased glucose response can imply peripheral tissue resistance to insulin or decreased pancreatic secretion of insulin (Ralston 2002).

In an insulin tolerance test, a set amount of insulin is administered (0.2-0.6 IU/kg) and the glucose response to this dose of insulin is measured (Firshman and Valberg 2007). Typically, the glucose level should decrease to 50% of the baseline level within approximately 20 to 30 minutes and return to baseline in 1.5 to 2 hours; insulin resistance is suggested by a decreased fall in glucose level as well as a quicker return to baseline levels.

A combined glucose-insulin test (CGIT) has been developed in the horse, and involves simultaneously administering a bolus of glucose and insulin (Eiler *et al.* 2005). Blood samples are collected up to 150 minutes after injection for measurement of blood glucose levels. The result is a characteristic 2-phase curve with a positive (hyperglycemia) and negative (hypoglycemia) phase. This curve was altered by administration of xylazine (for suppression of insulin secretion), stress, and certain disease states (e.g. Cushing's disease).

The euglycemic-hyperinsulinemic clamp technique (EHC) and the minimal model analysis of a frequently sampled intravenous glucose tolerance test (FSIGT) are the more well-established and commonly used quantitative tests for assessment of glucose and insulin dynamics

in the horse. The EHC involves inducing a state of hyperinsulinemia via a continuous insulin infusion, which in turn serves to suppress endogenous glucose production. The glucose concentration is clamped and held at basal concentrations by varying the rate of a continuous infusion of dextrose (Kronfeld *et al.* 2005a). During the latter part of the test, a steady state of euglycemia and hyperinsulinemia is reached and the glucose infusion rate is regarded as being equal to the quantity of glucose taken up by all tissues in response to exogenous insulin administration. This rate of whole body glucose uptake is designated as M. Another parameter known as M/I, which is an index of insulin sensitivity, is also calculated from the results of the EHC. M/I is a measure of the glucose uptake per unit of insulin. The disadvantages of the EHC are that it is labor intensive, requires some degree of technical expertise to perform, and is essentially inducing a nonphysiologic state (Kronfeld *et al.* 2005a).

The minimal model technique attempts to assess glucose and insulin dynamics in a more physiologic manner. It involves the use of a modified FSIGT, in which dextrose is administered followed by an insulin bolus 20 minutes after administration of the dextrose. The data is then applied to computerized mathematical modeling (minimal model method) in order to derive several parameters (Boston *et al.* 2003). The 3 main variables that are derived from the minimal model are the insulin sensitivity index (S_I), which is a measure of the ability of insulin to mediate glucose disposal, the glucose effectiveness (S_G), which is a measure of the ability of glucose to mediate its own disposal, and the AIR_g, which is the acute insulin response to glucose and is a measure of the responsiveness of pancreatic β cells to the glucose load (Kronfeld *et al.* 2005a). The disposition index (DI) is equal to $AIR_g \times S_I$ and indicates the appropriateness of the pancreatic β cell response relative to the degree of insulin resistance in the tissues. The main disadvantage of the minimal model method is that it may be influenced by endogenous insulin

secretion (Kronfeld *et al.* 2005a). One study comparing the EHC and minimal model method found that the EHC method was more repeatable (Pratt *et al.* 2005).

Proxies for evaluating insulin resistance from basal plasma glucose and insulin concentrations have been developed from the minimal model analysis results of healthy horses, and provide an easier and simpler method of quantifying glucose and insulin dynamics (Treiber *et al.* 2005b). The insulin sensitivity (SI) was estimated from the reciprocal of the square root of insulin (RISQUI), where SI = 7.93(RISQI) - 1.03. The AIR_g was estimated from the modified glucose-to-insulin ratio (MIRG), where AIR_g = 70.1(MIRG) - 13.8. The total predictive powers for RISQUI and MIRG were found to be 78% and 80%, respectively. Although significantly simpler and less time-consuming to perform, the proxies are not as accurate as either the clamping techniques or the minimal model analysis (Firshman and Valberg 2007).

The hyperglycemic clamp technique is another type of clamp analysis which evaluates the pancreatic β cell sensitivity to glucose (Rijnen and van der Kolk 2003). Glucose is administered initially as a bolus and then as a continuous rate infusion in order to reach a steady state of hyperglycemia. Hepatic glucose production is suppressed by the hyperglycemia. The derived parameters are M (rate of whole body glucose uptake), I (measurement of pancreatic β cell response to glucose), and M/I (index of sensitivity of tissues to insulin).

Parenteral nutrition in the horse

In equine medicine, parenteral nutrition is administered to critically ill patients and/or those patients who cannot ingest or tolerate feed in the digestive tract, with the goal being to prevent malnutrition. Malnutrition has been shown to be detrimental to overall immune function

as well as wound healing (Lopes and White 2002). In human medicine, malnutrition has been associated with an increased risk of morbidity in hospitalized patients. Concurrently, parenteral nutrition has been shown to reduce morbidity in malnourished patients (Jeejeebhoy 2001). Parenteral nutrition is generally administered more frequently to foals in comparison to adult horses partly because of the larger body size of the latter and subsequent increased financial costs. In addition, neonates have less body reserve and therefore are less tolerant of decreased energy intake. However, parenteral nutrition should be considered in any horse that cannot tolerate enteral intake, particularly when feed will be withheld for greater than 48-72 hours (Geor 2005). Parenteral nutrition in horses typically consists of a solution of dextrose and amino acids, or of dextrose, amino acids, and lipids. The dextrose and lipids provide energy, while the amino acids address protein requirements (Geor 2005). The three main solutions used to make up parenteral nutrition are 50% dextrose, 10% amino acids, and 20% lipids (Bercier 2003).

A study investigating parenteral nutrition in adult horses with gastrointestinal disease found that the most common reasons for parenteral nutrition supplementation were ileus, gastric reflux, and anorexia (Lopes and White 2002). The most common adverse effect was hyperglycemia, which in some cases necessitated temporarily reducing the infusion rate or administering a concurrent continuous intravenous insulin infusion. Another study assessing the effects of parenteral nutrition in adult horses following small intestinal resection and anastomosis found that treatment of horses post operatively with parenteral nutrition resulted in significantly lower serum triglycerides, total bilirubin, albumin, and urea, suggesting improved nutritional status (Durham *et al.* 2004). Horses treated with parenteral nutrition also had significantly higher concentrations of glucose and insulin. The authors concluded that this was due to some measure

of insulin resistance likely secondary to hypercortisolemia, and that these horses receiving parenteral nutrition post operatively were likely glucose intolerant.

Krause *et al.* performed a retrospective study examining parenteral nutrition in foals and found the reasons for administering parenteral nutrition were recumbency, depression, enterocolitis, and colic (Krause and McKenzie 2007). The most common complication associated with parenteral nutrition was hyperglycemia, which was addressed by decreasing the infusion rate of the parenteral nutrition, adding a continuous intravenous infusion of insulin, or monitoring hyperglycemia until it resolved. The occurrence of hyperglycemia in these foals was associated with a sepsis score > 11. Furthermore, the development of hyperglycemia and the need for an insulin infusion were associated with an increased likelihood of nonsurvival.

Little information is available in the literature regarding appropriate dosages of insulin to administer to horses in order to prevent the development of hyperglycemia. This is particularly true regarding the use of a continuous rate infusion of insulin. For foals, several values for initial infusion rates have been reported: (1) 0.01-0.02 IU/kg/hr (Bercier 2003), (2) 0.014-0.2 IU/kg/hr (Krause and McKenzie 2007), and (3) 0.07 IU/kg/hr (McKenzie and Geor 2007). The appropriate insulin infusion rate for adult horses is less clear and is not reported in current literature.

Conclusion

Therefore, the purpose of our study was to investigate appropriate insulin infusion rates in horses for the maintenance of euglycemia. Specifically, we planned on examining the effects of a continuous rate infusion of dextrose or dextrose and insulin on glucose and insulin dynamics in both healthy and endotoxin-exposed horses. Our hypothesis was that a continuous rate

infusion of insulin at a dosage of 0.07 IU/kg/hr would be effective in preventing the development of hyperglycemia in both healthy and endotoxin-exposed horses.

In human medicine, there has been an increasing awareness of the detrimental effects of hyperglycemia in hospitalized patients as well as the ability of insulin to attenuate these effects via strict glycemic control. Parenteral nutrition, while frequently a necessary component of supportive therapy, can exacerbate hyperglycemia in critically ill patients who are already predisposed to development of stress hyperglycemia. Intensive insulin therapy is routinely required concurrently with parenteral nutrition in order to prevent the development of hyperglycemia. While there has been a great deal of investigation into appropriate insulin dosages to use in humans, the same cannot be said for equine medicine. However, hospitalized equine patients, similar to their human counterparts, can also be prone to developing insulin resistance and hyperglycemia due to various disease states. Therefore, due to the many demonstrated benefits of insulin therapy, it stands to reason that further knowledge regarding insulin administration in patients with systemic inflammation would benefit the equine clinician in formulating more effective supportive treatment protocols for hospitalized patients.

Chapter 2. Glucose and insulin dynamics associated with continuous infusion of dextrose or dextrose and insulin in healthy and endotoxin-exposed horses

Introduction

In human medicine, critical care patients frequently require dextrose supplementation as a component of normal supportive therapy and care. An insulin infusion is often required in conjunction with the dextrose infusion in order to effectively maintain euglycemia. Conventional normalization of blood glucose levels has involved keeping the blood glucose concentration below 180-200 mg/dL, but an increasingly prevalent trend has been strict normalization of blood glucose concentrations, in which blood glucose concentration is kept under tighter control (Langouche and Van den Berghe 2006).

Many studies in human patients have shown numerous benefits in the use of intensive insulin therapy to maintain strict glycemic control (Grey and Perdrizet 2004; Krinsley 2004; Van den Berghe *et al*. 2005; van den Berghe *et al*. 2001). A landmark prospective study of 1548 patients in a surgical critical care unit showed a 34% reduction in mortality when blood glucose level was maintained between 80 -110 mg/dL (van den Berghe *et al*. 2001). Further benefits included a significant decrease in critical care complications, including a reduction in bloodstream infections, bacteremia, acute renal failure requiring dialysis or hemofiltration, critical illness polyneuropathy, dependence on mechanical ventilation, and prolonged inflammation. Subsequent studies have shown similar benefits in various subpopulations, including a reduction in hospital mortality, length of ICU stay, transfusion requirements, ventilator dependency, critical illness polyneuropathy, and total nosocomial infections (Grey and

Perdrizet 2004; Krinsley 2003; Van den Berghe *et al*. 2005). Furthermore, comparable benefits have been found with more moderate glycemic control. A study looking at a population of critically ill adult human patients found a decrease in hospital mortality of 29.3% and a decrease in length of ICU stay of 10.8%, as well as a reduced incidence of new renal insufficiency and requirement for red blood cell transfusions, when blood glucose was maintained lower than 140 mg/dL (Krinsley 2004).

Like their human counterparts, horses in a hospital setting often receive parenteral nutrition. Parenteral nutrition is used in equine practice to provide energy and nutritional support to critically ill patients as well as those who cannot ingest food or tolerate or digest feed in the gastrointestinal tract. The aim is to prevent malnutrition and catabolism, particularly as malnutrition can deleteriously affect immune function and wound healing (Lopes and White 2002). Malnutrition has been shown in human medicine to increase the risk of morbidity in hospitalized patients, while parenteral nutrition has in turn been shown to reduce morbidity in malnourished patients (Jeejeebhoy 2001). Parenteral nutrition should be considered in any horse that cannot tolerate enteral feeding, especially when oral feed is expected to be withheld for greater than 48-72 hours (Geor 2005).

An insulin infusion run concurrently with the parenteral nutrition may also be a requirement in order to prevent the development of hyperglycemia, particularly as horses, similar to humans, can become insulin resistant due to various conditions. These conditions include endotoxemia, pituitary adenoma, hyperlipidemia, obesity, a diet high in sugar and starch, and pregnancy (Fowden *et al*. 1984; Hoffman *et al*. 2003; Kronfeld *et al*. 2005a; Kronfeld *et al*. 2005b; Treiber *et al*. 2005a). The increasing recognition of the numerous benefits of preventing hyperglycemia through the use of intensive insulin therapy in human medicine has in turn

emphasized the importance of following a similar protocol in veterinary medicine as well. However, little information is available regarding appropriate dosages of insulin to administer to horses to prevent hyperglycemia. This is especially true regarding the use of a continuous rate infusion of insulin, which in human medicine has been found to result in more effective glycemic control and reduced mortality in comparison to using intermittent subcutaneous administration of insulin (Furnary *et al*. 2003; Meijering *et al*. 2006).

The objective of this study was to investigate and characterize the effects of a continuous rate infusion of dextrose or dextrose and insulin on glucose and insulin dynamics in both healthy and endotoxin-exposed horses. Administration of a low dose of endotoxin has been used in horses to mimic the clinicopathologic changes seen in endotoxemia, including the development of an inflammatory response. This low-dose endotoxin model is well-established in the horse and results in fairly consistent and reproducible changes in clinical, hematological, and hemodynamic parameters (Barton *et al*. 1998; Bueno *et al*. 1999; Clark *et al*. 1991; Clark and Moore 1989; Danek 2006; Tetens *et al*. 2004; Toribio *et al*. 2005; Winchell *et al*. 2002). Our hypothesis was that a continuous rate infusion of insulin at a rate of 0.07 IU/kg/hr would prevent the development of hyperglycemia induced by the administration of dextrose in both healthy and endotoxin-exposed horses.

Materials and Methods

Horses

Nine healthy mature Thoroughbred mares were used in the study. Horses ranged from 4 to 18 years old (mean ± SD, 11 ± 4.81 years) and weighed between 457 and 601 kg (mean ± SD, 531 ± 43 kg). Body condition scores were not directly assessed; however, the horses all subjectively appeared to be in similar body condition. The horses were maintained in one group on pasture turnout with access to fresh water at all times. The study was conducted at the Middleburg Agricultural Research and Extension (MARE) center. The protocol was approved by the Virginia Tech Institutional Animal Care and Use Committee.

Saline and dextrose infusions

The first part of the study was designed to investigate the effects of a continuous dextrose infusion on glucose and insulin dynamics in healthy horses. Eight mares were used in a balanced crossover design. The two treatments were control (saline) solution (Treatment 1) and dextrose (Treatment 2), with horses randomly assigned to initial treatment (4 horses initially received saline solution and 4 horses initially received dextrose solution). After a 3-4 day washout period, the experiment was repeated with horses receiving the other treatment. On the day prior to each experimental session a catheter[1] was aseptically placed into each jugular vein and secured to the skin with cyanoacrylate. One catheter was used for dextrose or saline infusion and the other

[1] Abbocath-T 14G X 5½", Hospira, Lake Forest, IL

catheter was used for collection of blood samples for measurement of plasma glucose and insulin concentrations.

Blood samples were collected 15 minutes and 5 minutes before beginning the infusion for determination of baseline concentrations of plasma glucose and insulin. Then a dextrose solution (20% dextrose) was administered for 6 hours as a continuous rate infusion at the calculated rate needed to deliver 30 kcal/kg/day, which was equivalent to a rate of 367.6 mg/kg/hour. Alternatively, saline was delivered as a constant rate infusion at the equivalent rate. Solutions were administered using a volumetric delivery pump.[2] Blood samples were collected at 1, 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 minutes after initiation of dextrose or saline administration for measurement of plasma glucose and insulin concentrations. At each sampling time ten mL of blood were withdrawn from the catheter and discarded, then another 10 mL of blood was collected for analysis. Immediately after collection, the catheter was flushed with 10 mL of heparinized saline to maintain patency. Horses were housed in box stalls during the experiment and given free choice access to grass hay and water.

Dextrose and insulin infusions

The second part of the study was designed to investigate the effects of a continuous dextrose and insulin infusion on glucose and insulin dynamics in both healthy and endotoxin-exposed horses. This portion of the study was performed beginning one week after the conclusion of the first portion of the study, which provided for a one week washout period. Seven of the eight mares used in the first part of the study were used again in the second part of the study, with one mare being replaced due to the identification of an unexpected pregnancy. In

[2] Alaris Imed Gemini PC-2, Alaris Medical Systems, San Diego, CA

total, eight mares were used in a balanced crossover design. The two treatments of the crossover consisted of a treatment in which the mares received a low dose of lipopolysaccharide (LPS) to induce a transient state of insulin resistance (Treatment 4) or a treatment where the mares did not receive LPS (Treatment 3). The eight horses were randomly assigned to initial groups (4 horses initially receiving LPS and 4 horses initially not receiving LPS). After a 5-8 day washout period, the experiment was repeated with each group of horses receiving the alternate treatment. On the day prior to each experimental session a catheter[a] was placed into each jugular vein and secured with cyanoacrylate. One catheter was used for dextrose or saline infusion and the other catheter was used for collection of blood samples for measurement of plasma glucose and insulin concentrations. After a washout period, the experiment was repeated with horses being placed into the other group and receiving the opposite treatment.

The LPS solution used in the study was formulated by adding 20 mL of 0.9% sterile saline solution to 100 mg of lyophilized *Escherichia coli* O55:B5 LPS.[3] One mL of the resulting stock solution was then added to 999 mL of 0.9% sterile saline to make up a final amount of 1 liter of 5 μg/mL concentration LPS solution. The calculated dose of LPS solution was added to 1 liter of 0.9% sterile saline and administered to the horses (35 ng/kg intravenously over 30 minutes) using a volumetric delivery pump[b] 24 hours prior to the administration of the dextrose and insulin infusions. Blood samples were collected immediately prior to LPS infusion, 3 hours after LPS infusion, and 24 hours after LPS infusion for determination of complete blood counts (CBC) and serum TNF-α concentration. Horses were housed in box stalls during LPS infusion

[3] *Escherichia coli* serotype O55:B5 LPS, Sigma-Aldrich Corporation, St Louis, MO

and given free choice access to grass hay and water. Attitude and vital parameters were assessed every 15 minutes for 6 hours after LPS administration.

Blood samples were collected 15 minutes and 5 minutes before beginning the dextrose and insulin infusions for determination of baseline concentrations. Then a dextrose solution (20% dextrose) was administered for 6 hours as a continuous rate infusion at the calculated rate needed to deliver 30 kcal/kg/day. A continuous rate infusion of insulin[4] was simultaneously administered at the rate of 0.07 IU/kg/hr. The amount of insulin that would be required for the 6 hour infusion was calculated and added to 1 liter of 0.9% sterile saline. Ten mL of blood was drawn from each horse and placed in a glass tube with no additives. The blood was allowed to clot at room temperature and the serum harvested. The serum was then added to the insulin solution to help prevent adherence of the insulin to the fluid administration lines. Solutions were administered using a volumetric delivery pump.[b] Blood samples were collected at 1, 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 minutes (and in half of the cases, 390, 420, and 450 minutes) after initiation of the dextrose and insulin infusions for measurement of plasma glucose and insulin concentrations. Ten mL of blood were withdrawn from the catheter and discarded, then another 10 mL of blood was collected for analysis. Immediately after collection, the catheter was flushed with 10 mL of heparinized saline to maintain patency. Horses were housed in box stalls during the experiment and given free choice access to grass hay and water. Blood glucose concentration was measured every 30 minutes throughout the experiment using a handheld glucometer.[5] If the blood glucose concentration was measured as < 50 mg/dL, a bolus of 125 mL of 50% dextrose was administered and this was repeated as needed every 30 minutes until the blood glucose concentration was > 50 mg/dL. If blood glucose concentration exceeded

[4] Humulin-R, Eli Lilly and Company, Indianapolis, IN
[5] Ascensia Contour Blood Glucose Meter, Bayer HealthCare, Tarrytown, NY

> 350 mg/dL, the insulin infusion rate was increased by 0.07 IU/kg/hr and repeated as needed every 1 hour until blood glucose concentration was < 350 mg/dL. At the end of the experiment, if blood glucose concentration was ≥ 120 mg/dL, both dextrose and insulin infusions were discontinued. If blood glucose concentration was < 120 mg/dL, only the insulin infusion was discontinued. The dextrose infusion was discontinued after 1 hour and/or when euglycemia was achieved. For the purposes of our study, we defined hyperglycemia as a blood glucose concentration > 180 mg/dL.

Analysis of samples

Blood samples for measurement of plasma glucose and insulin concentrations were placed into evacuated tubes that contained lithium heparin and immediately placed in an ice bath. They were centrifuged for 10 minutes at 3,000 X *g* within 30 minutes of collection, after which the plasma was harvested and stored at -20ºC for 24 hours and later at -80ºC until analysis. Samples for complete blood counts were placed into evacuated tubes that contained EDTA and were immediately processed. Blood samples for TNF-α concentrations were collected into glass tubes with no additives and allowed to clot at 4ºC overnight. Serum was then harvested and stored at -20ºC until analysis. Plasma glucose concentrations were measured by using an automated analyzer that determines the glucose concentration via a timed endpoint method utilizing the hexokinase reaction and subsequent monitoring of the change in absorbance.[6] Plasma insulin concentrations were measured using a commercially available radioimmunoassay[7] previously validated for equine plasma and serum. Complete blood counts

[6] Beckman Synchron CX5 Delta Clinical System, Beckman Coulter Inc, Fullerton, CA
[7] Coat-A-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA

were determined by use of an automated analyzer.[8] TNF-α concentrations were measured using a commercially available equine-specific ELISA.[9]

Statistical analysis

Values were expressed as mean ± SD. Glucose and insulin data were log transformed to achieve normal distributions. A two-way repeated measures ANOVA was used to compare blood glucose and plasma insulin concentrations during Treatment 1, Treatment 2, Treatment 3, and Treatment 4. Posthoc analysis was performed using the Tukey HSD test. A two-way repeated measures ANOVA was used to compare temperature, heart rate, and respiratory rate over time during the LPS infusion. Posthoc testing was performed with the Tukey HSD test. Serum TNF-α concentration data was analyzed using the Mann-Whitney U test. CBC data was analyzed using a paired t-test. Analyses were performed using two computerized statistical packages: Statistica 6.0 and Minitab 13. Results were considered significant at a value of $P \leq 0.05$.

[8] Abx Pentra 60 Cell Counter, Horiba ABX Diagnostics, Montpellier, France
[9] Equine TNFα ELISA Screening Set, Endogen, Rockford, IL

Results

The blood glucose and plasma insulin concentrations observed in all of the four treatment phases are provided in tables 1 and 2.

Sample Time	Treatment 1	Treatment 2	Treatment 3	Treatment 4
-15	105.0 ± 5.1	106.6 ± 2.9	101.9 ± 0.5	108.1 ± 3.2
-5	103.0 ± 3.2	106.7 ± 2.4	101.7 ± 0.3	108.9 ± 3.5
1	103.1 ± 4.5	112.8 ± 1.8	109.9 ± 1.2	114.3 ± 3.1
2	103.5 ± 3.7	120.1 ± 2.3	114.6 ± 1.8	120.2 ± 3.3
5	104.3 ± 3.5	132.8 ± 2.6	127.9 ± 2.5	133.9 ± 3.9
10	106.0 ± 3.7	150.7 ± 3.7	140.5 ± 2.1	151.0 ± 5.6
15	106.1 ± 3.6	167.3 ± 6.6	144.5 ± 3.1	157.8 ± 7.1
30	106.7 ± 3.7	181.2 ± 16.9	129.7 ± 4.7	160.8 ± 13.2
45	106.5 ± 4.0	212.7 ± 8.3	115.5 ± 7.3	159.9 ± 17.3
60	108.8 ± 2.9	222.5 ± 11.0	105.6 ± 8.3	152.8 ± 19.7
90	107.7 ± 3.5	224.1 ± 20.2	110.7 ± 7.7	148.3 ± 21.1
120	106.3 ± 4.9	218.8 ± 25.7	119.9 ± 13.0	157.3 ± 20.9
150	105.7 ± 6.3	217.1 ± 26.6	122.3 ± 13.8	154.6 ± 25.3
180	107.0 ± 6.0	220.6 ± 23.2	127.8 ± 14.9	160.1 ± 25.6
240	108.1 ± 4.6	204.9 ± 23.8	128.5 ± 15.2	173.5 ± 23.7
300	105.7 ± 5.4	184.4 ± 21.3	134.0 ± 16.5	166.1 ± 21.8
360	107.8 ± 4.9	180.3 ± 20.3	135.2 ± 15.7	174.5 ± 18.3

Table 1. Blood glucose concentrations in mg/dl (mean±SD) in all four treatment phases over time. Treatment 1 = saline infusion; Treatment 2 = dextrose infusion; Treatment 3 = dextrose and insulin infusion; Treatment 4 = dextrose and insulin infusion following LPS administration

Sample Time	Treatment 1	Treatment 2	Treatment 3	Treatment 4
-15	9.9 ± 2.9	9.7 ± 1.5	11.7 ± 2.4	14.8 ± 3.0
-5	9.7 ± 4.2	11.2 ± 2.1	11.6 ± 2.5	15.8 ± 4.1
1	10.3 ± 3.1	11.3 ± 1.7	13.1 ± 3.1	16.6 ± 3.5
2	9.9 ± 3.2	19.5 ± 3.8	29.1 ± 4.6	26.8 ± 4.9
5	10.1 ± 3.0	20.7 ± 3.0	43.7 ± 7.5	46.8 ± 5.5
10	11.1 ± 3.5	24.7 ± 2.9	59.4 ± 10.1	69.9 ± 7.5
15	11.9 ± 3.6	24.7 ± 3.6	75.1 ± 10.4	83.2 ± 7.3
30	13.5 ± 4.2	25.8 ± 5.2	86.5 ± 12.6	107.5 ± 17.2
45	12.0 ± 5.5	32.5 ± 3.6	95.7 ± 12.3	125.7 ± 23.1
60	13.1 ± 4.5	36.0 ± 3.8	98.7 ± 14.8	121.5 ± 24.2
90	12.1 ± 3.2	39.4 ± 5.9	111.0 ± 20.7	137.9 ± 28.3
120	12.0 ± 3.3	47.7 ± 9.9	125.9 ± 27.5	163.4 ± 41.6
150	10.6 ± 2.6	54.8 ± 10.9	134.0 ± 32.4	173.1 ± 45.6
180	11.8 ± 3.2	60.7 ± 12.3	151.5 ± 40.7	191.0 ± 54.1
240	9.9 ± 2.9	66.2 ± 17.6	174.7 ± 50.6	228.3 ± 63.9
300	9.8 ± 2.6	88.0 ± 25.6	202.8 ± 63.0	253.1 ± 82.0
360	9.8 ± 2.4	73.3 ± 17.2	187.1 ± 49.4	271.0 ± 71.8

Table 2. Insulin concentrations in mU/L (mean±SD) in all four treatment phases over time. Treatment 1 = saline infusion; Treatment 2 = dextrose infusion; Treatment 3 = dextrose and insulin infusion; Treatment 4 = dextrose and insulin infusion following LPS administration

Saline and Dextrose Infusions

The saline infusion (Treatment 1) was intended to serve as a control for the potential effects of the environment, fluid infusion protocol, and the sampling protocol on the endogenous production of insulin and regulation of normoglycemia. Blood glucose concentrations were significantly greater with Treatment 2 as compared to Treatment 1, with posthoc analysis showing significant differences at times 10 minutes to 360 minutes. Insulin concentrations were also significantly greater with Treatment 2, with significance at 10 minutes and then from times 45 minutes to 360 minutes.

LPS Infusion

The purpose of the infusion of a low dose of LPS was to induce a transient state of insulin resistance. Clinical signs associated with LPS infusion were fairly mild and resolved by the end of the 6 hour observation period, and included an increase in rectal temperature, mild tachycardia, trembling, mild signs of colic, and soft manure. At least one of these signs was present in each horse following LPS infusion. Temperature, heart rate, and respiratory rate were all significantly different over time (P=0.0000). However, only temperature was found to be significant with post-hoc comparisons (P<0.05 from times 150 minutes to 345 minutes). White blood cell counts were significantly decreased (P=0.00) three hours following LPS administration ($4.7x10^3/\mu L \pm 0.5x10^3/\mu L$) as compared to the samples obtained immediately prior to endotoxin administration ($7.4x10^3/\mu L \pm 1.0x10^3/\mu L$). Serum TNF-α concentrations were significantly elevated (P= 0.05) three hours following LPS administration (median 229 pg/ml

(range: 103-2380)) as compared to immediately prior to the administration of endotoxin (median 65 pg/ml (range: 0-933)).

Dextrose and Insulin Infusion

The dextrose infusion (Treatment 2) from the first part of the study was intended to serve as a control for the second part, as it demonstrated glucose and insulin responses to a constant rate infusion of dextrose without an accompanying insulin infusion. The insulin and dextrose infusion without prior administration of LPS (Treatment 3) represents the control for the effects of LPS (Treatment 4). When comparing Treatment 2 to Treatment 3, it was found that Treatment 2 was associated with higher blood glucose values, with significance at times 45 minutes to 240 minutes. Treatment 3 was associated with higher insulin values, with significance at times 15, 30, and 45 minutes.

When Treatment 2 was compared to Treatment 4, there was no significant difference in blood glucose concentrations over time. Insulin concentrations were significantly greater with Treatment 4 from times 10 minutes to 360 minutes. When Treatment 3 and Treatment 4 were compared the blood glucose and insulin concentrations were persistently higher with Treatment 4 from the 15 minutes until the end of the protocol, however the differences were not great and significance was not reached at any time point.

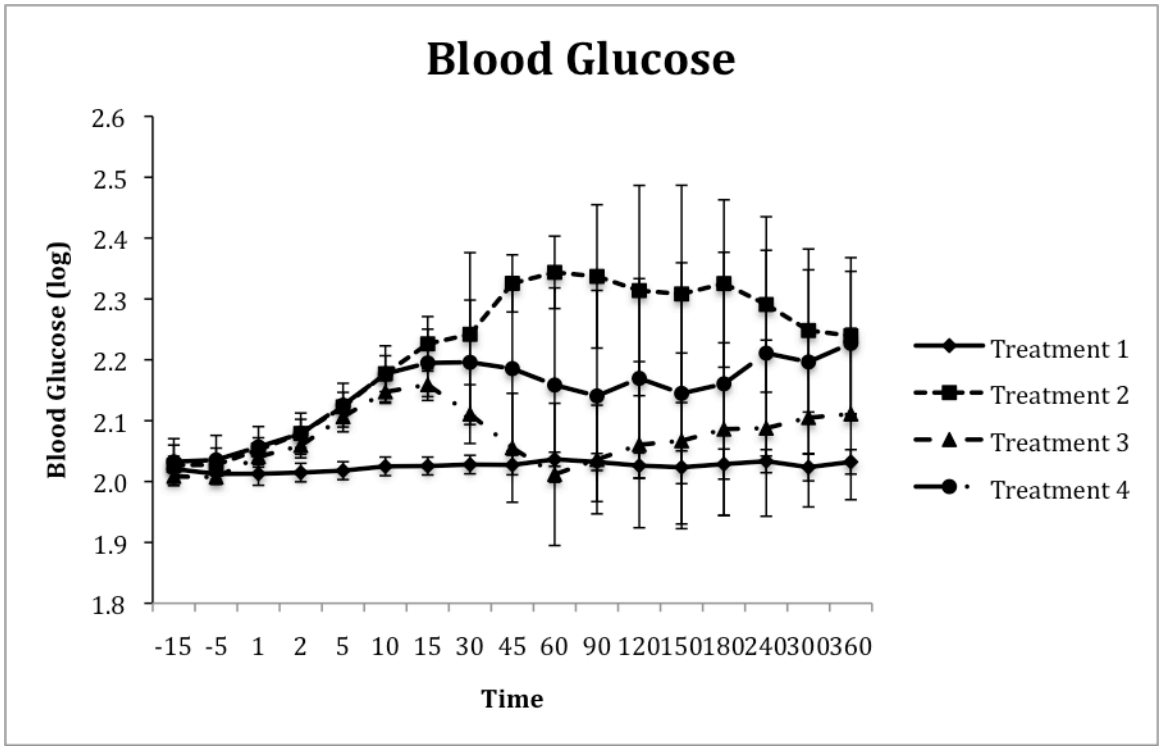

Figure 2. Log of blood glucose concentrations for all four treatment groups over time. Treatment 1 = saline infusion; Treatment 2 = dextrose infusion; Treatment 3 = dextrose and insulin infusion; Treatment 4 = dextrose and insulin infusion following LPS administration

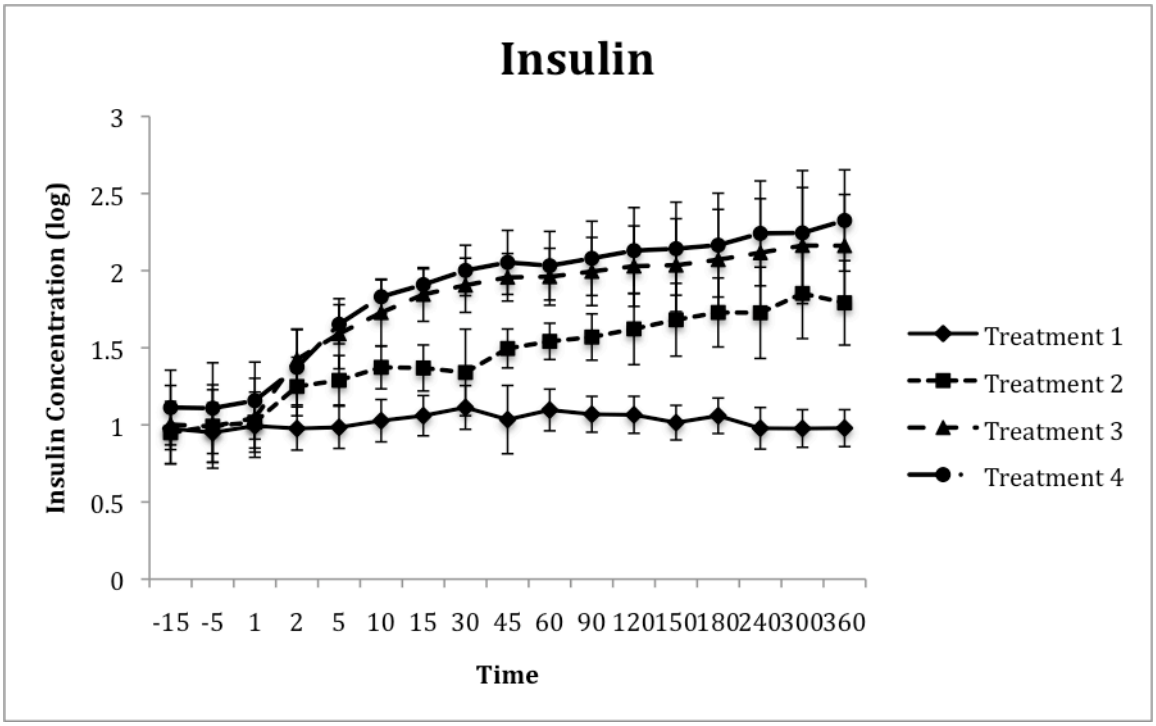

Figure 3. Log of plasma insulin concentrations for all four treatment phases over time. Treatment 1 = saline infusion; Treatment 2 = dextrose infusion; Treatment 3 = dextrose and insulin infusion; Treatment 4 = dextrose and insulin infusion following LPS administration

Discussion

A continuous rate infusion of insulin at a dose of 0.07 IU/kg/hr was shown in our study to prevent the development of hyperglycemia associated with a dextrose infusion, with this effect being demonstrated in both healthy horses and endotoxin-exposed horses. A dextrose infusion rate equivalent to administration of 30 kcal/kg/day was selected for this study. The mean daily digestible energy requirement for horses at maintenance is estimated to be 30.3 kcal/kg. This corresponds to horses with a sedentary lifestyle, for example, those that are confined or have a quiet temperament (Council 2007). The energy requirements of hospitalized horses may be reduced as a result of decreased energy consumption, or alternatively may be increased in cases of critical illness and disease states such as sepsis (Geor 2005). Therefore, due to the wide variation that is likely present among hospitalized patients, as well as the difficulty of measuring true energy expenditure, we chose a rate equivalent to 30 kcal/kg/day as an average representative value and a reasonable starting point for energy requirements.

Development of hyperglycemia has been reported as the most common complication of parenteral nutrition in hospitalized adult horses (Durham *et al*. 2004; Lopes and White 2002) and foals (Krause and McKenzie 2007), with a concurrent insulin infusion being required in some cases for control of the hyperglycemia. There is little information available in the literature regarding an appropriate initial insulin infusion rate, particularly in adult horses. In foals, several rates have been reported: (1) 0.01-0.02 IU/kg/hr (Bercier 2003), (2) 0.014-0.2 IU/kg/hr (Krause and McKenzie 2007), and (3) 0.07 IU/kg/hr (McKenzie and Geor 2007). We chose an insulin infusion rate of 0.07 IU/kg/h based on our clinical success with this dose as a reasonable starting

dose. This dose appears to be generally well tolerated in both adult horses and foals in our clinical experience.

Saline infusion resulted in no significant changes in the plasma glucose or insulin concentrations at any time point. Intravenous infusion of a 20% dextrose solution at a rate equivalent to administering 30 kcal/kg/day resulted in hyperglycemia for nearly the entire study period. Blood glucose concentration was greater than 180 mg/dL from time 30 minutes to 300 minutes. Insulin concentration also increased during the dextrose infusion, resulting in peak concentrations of 88.0 ± 25.6 mU/L at 300 minutes. This increase in insulin concentration may have reflected endogenous insulin secretion from pancreatic β cells in response to an exogenous glucose load and is indicative of an expected endocrine response. Alternatively, the increase in insulin concentration may have been due to decreased hepatic clearance of insulin. A possible confounding factor for this first part of the study was the fact that one of the mares was found to be unexpectedly pregnant. Pregnancy in the mare has been found to be associated with increased pancreatic β cell sensitivity to endogenous and exogenous glucose, increased insulin degradation, and insulin resistance (Fowden *et al*. 1984). Therefore, it is possible that this mare's response to an exogenous glucose load was altered by the pregnancy, but no significant differences were observed in this individual when compared to the rest of the group. The mare was eliminated from further participation in the study due to concerns regarding the safety of the fetus during or after the induction of experimental endotoxemia.

This study was comprised of two main parts, with each part consisting of a balanced crossover design between two treatments. The two treatments in the first part of the study were saline and dextrose, while the treatments in the second part of the study were dextrose and insulin without LPS administration and dextrose and insulin with LPS administration. In total,

there were four treatments for the entire study. Conceivably, the design of the study could have been improved by utilizing a protocol that incorporated all four treatments into one balanced crossover design. However, we considered it reasonable to directly compare all the treatments to one another, as all treatments were performed during the same month with the same group of horses (barring the one horse that was replaced due to unexpected pregnancy).

Insulin concentrations were significantly greater over time with Treatment 3 versus Treatment 2. This increase was likely due to the exogenous administration of insulin. The increased insulin concentrations in turn resulted in maintenance of euglycemia (blood glucose <180 mg/dL) throughout the study period in the Treatment 3 group, in contrast to the persistent hyperglycemia that occurred in the Treatment 2 group. These results lend support to the dose of 0.07 IU/kg/hr as a valid starting point for a continuous rate infusion of insulin. Interestingly, there was a noticeable decrease in blood glucose in the Treatment 3 group at 60 minutes, which corresponds with what has been observed clinically and may reflect saturation of insulin receptors and maximal effect of insulin.

Infusion of a low dose of endotoxin (20-35 ng/kg) in the horse results in consistently reported changes in hematologic, hemodynamic, and physical variables. Clinical signs include increases in heart rate, respiratory rate, and rectal temperature, as well as mild signs of colic and depression. Hematologic changes include leukopenia with or without neutropenia, as well as a rebound leukocytosis and neutrophilia in some studies (Bueno *et al*. 1999; Clark *et al*. 1991; Clark and Moore 1989; Tetens *et al*. 2004; Toribio *et al*. 2005; Winchell *et al*. 2002). An increase in serum IL-6 and TNF-α concentrations has also been demonstrated (Bueno *et al*. 1999). This low-dose endotoxin model has been successfully used in various studies to test the efficacy of pharmacologic agents in ameliorating the changes induced by endotoxemia (Barton *et*

al. 1998; Danek 2006; Tetens *et al*. 2004). The changes observed after endotoxin infusion in the horses in this study, including changes in clinical parameters, white blood cell counts, and serum TNF-α concentrations, were consistent with what has been reported, and as expected, were transient in nature. Therefore, successful induction of a temporary state of inflammation and insulin resistance in these horses was assumed. However, the presence of insulin resistance was not definitively confirmed by quantitative testing. Furthermore, investigation of glucose and insulin dynamics in response to the low dose endotoxin model appear to vary, with one study finding a reduction in insulin sensitivity at 24 hours after endotoxin challenge (Toth *et al*. 2008), while another study found a transient increase in insulin sensitivity at 6 hours, followed by a decrease in insulin sensitivity at 24 hours after LPS administration (Vick *et al*. 2008). In the latter study, administration of LPS was also found to be associated with increased expression of inflammatory cytokines such as TNF-α and IL-6 in both blood and adipose tissue.

When comparing Treatment 3 and Treatment 4, the insulin concentrations were consistently higher in the Treatment 4 group from times 10 minutes to 360 minutes, although significance was not achieved. The increased insulin concentrations in the Treatment 4 group likely reflect increased endogenous pancreatic secretion of insulin due to induction of an insulin resistant state. Increased insulin concentrations could also be attributed to decreased hepatic clearance. The blood glucose concentrations also were consistently higher in the Treatment 4 group from times 15 minutes to 360 minutes, although significance was not achieved. The fact that blood glucose concentrations were higher in the Treatment 4 group despite the increase in insulin concentration relative to Treatment 3 further supports the development of insulin resistance in response to LPS administration. Despite the presumed induction of insulin

resistance, euglycemia was present in the Treatment 4 group throughout the study period, ranging between 140 to 180 mg/dL from time 10 minutes to 360 minutes.

These results serve to validate the dose of insulin used in this study in regards to effective prevention of hyperglycemia when dextrose is infused at a rate of 367 mg/kg/hr. Administration of dextrose to healthy horses resulted in hyperglycemia despite the expected response of increased endogenous insulin secretion. Concurrent administration of an insulin infusion with the dextrose infusion prevented the development of hyperglycemia, even in endotoxin-exposed horses. This insulin dose was also demonstrated to be safe, as hypoglycemia did not occur in any of the horses in either Treatment 3 or Treatment 4 during the combined dextrose and insulin infusion. For the purposes of this study, we defined hyperglycemia as a blood glucose concentration > 180 mg/dL. However, further research should include investigation of stricter glycemic control, as many of the benefits of insulin therapy in human medicine have involved fairly tight control of blood glucose. Our hypothesis, that a continuous rate infusion of insulin at a dosage of 0.07 IU/kg/hr would prevent the development of hyperglycemia induced by the administration of dextrose in both healthy and endotoxin-exposed horses, was confirmed by the results obtained in this study.

The prevention of hyperglycemia, rather than the infused insulin dose *per se,* appears to be largely responsible for the observed reductions in mortality and morbidity in critically ill human patients (Van den Berghe *et al*. 2003). Hyperglycemia is a frequent occurrence in critically ill patients. This is due to the peripheral insulin resistance and increased hepatic output of glucose (hepatic insulin resistance), developments which are mediated by increased levels of catecholamines, cortisol, growth hormone, glucagon, and inflammatory cytokines (Langouche and Van den Berghe 2006; Marik and Raghavan 2004; McCowen *et al*. 2001b). Other factors

that exacerbate the stress hyperglycemia include administration of parenteral or enteral nutrition, corticosteroids, and dialysis solutions, as well as bed rest, which decreases skeletal muscle sensitivity to insulin (Krinsley 2003; Stuart *et al*. 1988). Hyperglycemia at admission and throughout the hospital stay has been associated with increased mortality and morbidity. One retrospective study examining 1826 intensive care patients found that even a modest degree of hyperglycemia resulted in a significantly increased mortality rate (Krinsley 2003). This effect has been demonstrated in various sub-populations of patients, including those with acute myocardial infarction, severe brain injury, trauma patients, and pediatric critically ill patients (Faustino and Apkon 2005; Jeremitsky *et al*. 2005; Laird *et al*. 2004; Rovlias and Kotsou 2000; Sala *et al*. 2002; Yendamuri *et al*. 2003). Hyperglycemia leads to impaired immune function through various mechanisms, including decreased polymorphonuclear neutrophil function, inhibition of intracellular bactericidal and opsonic activity, reduction in polymorphonuclear leukocyte and alveolar macrophage respiratory burst, and inactivation of immunoglobulins via glycosylation (Langouche and Van den Berghe 2006; Marik and Raghavan 2004; McCowen *et al*. 2001b; Vanhorebeek *et al*. 2005b; Vanhorebeek *et al*. 2006b). The detrimental effects of hyperglycemia have not been as thoroughly explored in horses, but higher blood glucose levels have been found to be associated with nonsurvival in horses with colic (Parry *et al*. 1983) and with acute abdominal disease (Hollis *et al*. 2007). In the latter study, it was found that nonsurvival was associated with higher mean blood glucose concentrations at admission, and at 24, 36, and 48 hours after admission, as well as higher maximum and minimum concentrations in the first 24 hours after admission.

Intensive insulin therapy to prevent hyperglycemia attenuates many of the aforementioned negative effects. In addition to glycemic control in critically ill patients, insulin

therapy also appears to have other nonglycemic beneficial effects. These include reversal of hypertriglyceridemia and general correction of dyslipidemia, improvement of myocardial function and protection of the myocardium during acute myocardial infarction, prevention of endothelial dysfunction, reduction in inflammatory cytokines and mediators, and protection of the hepatocytic mitochondria (Vanhorebeek *et al*. 2005a; Vanhorebeek *et al*. 2005b). This raises the possibility of similarly using insulin therapy in equine patients for correction of hypertriglyceridemia and dyslipidemia. A condition known as severe hypertriglyceridemia has been demonstrated in equine patients, with the criteria including a serum triglyceride concentration greater than 5.65 mmol/l and no visible lipemia (Dunkel and McKenzie 2003). Furthermore, all the horses in Dunkel's report were found to have clinical and laboratory findings supportive of systemic inflammatory response syndrome. The hypertriglyceridemia was successfully treated in these cases with an intravenous dextrose and/or partial parenteral nutrition infusion; however, based on the findings in human medicine, it is possible that incorporating an insulin infusion into the treatment regimen in these horses would improve and enhance the resolution of hypertriglyceridemia. Further work would have to be done to investigate this possibility in horses.

Due to the many demonstrated benefits, strict normalization of blood glucose levels using intensive insulin therapy is becoming widely accepted as a prerequisite for providing effective care to hospitalized patients. In fact, many intensive care units have begun implementing nomograms in order to standardize intensive insulin therapy. This involves designing a standard protocol that is to be used as a guide by hospital staff to make adjustments to the rate of insulin infusion based both on a patient's current blood glucose level and current rate of insulin infusion. This is in contrast to a more conventional approach, in which an arbitrary decision would be

made by the clinician in regards to how the rate of insulin infusion should be adjusted. Recent studies have shown that standardization of intensive insulin therapy improves efficiency, both by decreasing the time to reach the target glucose range and by increasing the time spent within the target range. It also increases safety by reducing the incidence of severe hypoglycemia and the frequency that "rescue" dextrose must be administered or that the insulin infusion must be temporarily discontinued (Brown and Dodek 2001; Kanji *et al*. 2004; Krinsley 2004). No such nomograms have been developed for equine patients to date.

In this study, an insulin infusion rate of 0.07 IU/kg/hr administered concurrently with a dextrose infusion was effective in preventing hyperglycemia, even after administration of LPS. These findings provide a useful starting point in the determination of appropriate insulin infusion protocols to be utilized in the horse. Further research would include application of the results of this study to investigate glucose and insulin dynamics associated with continuous infusion of dextrose and insulin in hospitalized horses.

Conclusion

Administration of a continuous rate infusion of insulin at 0.07 IU/kg/hr is effective in preventing hyperglycemia in horses receiving a concurrent infusion of dextrose. This is the case for both healthy horses and for horses that have received a low dose of LPS in order to induce a transient inflammatory state mimicking that of endotoxemia. This dose of insulin also appears safe to use, as no incidents of hypoglycemia developed during the infusion in any of the study horses. Further research is indicated to determine appropriate dosages of insulin for stricter glycemic control. In addition, the effect of a continuous rate infusion of insulin on glucose and

insulin dynamics should be investigated in hospitalized horses requiring parenteral nutrition, as these are likely to differ from what was found in the healthy horses in our study.

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